

Solid State Physicochemical Study of Chlorophyll *a* Derivatives and Their Glycol Conjugates

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*This study focuses on solid state physico-chemical properties of recently synthesized potential sensitizers for photodynamic therapy developed on a chlorophyll *a* platform. Pheophorbide *a* and chlorin *e*₆ derivatives as well as two their glycol conjugates have been synthesized and identified via UV-Vis, NMR and mass spectra. The behavior of photosensitizers (PSs) in a solid state has been studied. They were found to be stable in inert atmosphere at least up to 520 K, the thermal stability decreases from phlorin to chlorin molecules and, especially, after their glycol substitution. The thermodestruction of macroheterocycles takes place after their melting. Both glycol conjugates form intramolecular H-bonds in two different conformations, what is confirmed by phase transitions on DSC curves and quantum-chemical analysis data.*

Keywords: Chlorophyll *a*, photosensitizers, phase transitions, thermal stability, intramolecular H-bonding.

Физико–химические свойства производных хлорофилла *a* и их диэтиленгликолевых конъюгатов в твердой фазе

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*Производные феофорбида *a* и хлорина *e*₆, а также их конъюгаты с диэтиленгликолем были синтезированы и идентифицированы спектральными (ЭСП, ЯМР ¹H, MS) методами. Изучено поведение фотосенсибилизаторов (ФС) в твердой фазе. Соединения стабильны в инертной атмосфере до 520 K и выше, их термическая устойчивость снижается при переходе от флоринов к хлоринам, особенно после введения в молекулу гликольных фрагментов, а само разрушение всегда следует за процессом плавления. Из данных ДСК и квантово-химического анализа следует, что оба гликольных производных образуют внутримолекулярные Н-связи, формируя два типа конформаций.*

Ключевые слова: Хлорофилл *a*, фотосенсибилизаторы, фазовые переходы, термическая устойчивость, внутримолекулярные Н-связи.

(2H, m, H^{17} , H^{18}), 3.84 (3H, s, $15-CH_2COOCH_3$), 3.62 [3H, s, $17-CH_2CH_2COOCH_3$], 3.56 (3H, s, $12-CH_3$), 3.50 (3H, s, $2-CH_3$), 3.33 (3H, s, $7-CH_3$), 3.81 (2H, q, $J=10.0$ Hz, $8-CH_2CH_3$), 1.90–2.60 [4H, m, $17-(CH_2CH_2COOCH_3)$], 1.73 (6H, m, $18-CH_3$, $8-CH_2CH_3$) –1.60 (br.s, ^{21}NH), –1.80 (br.s, ^{23}NH).

MS-spectra were recorded on Thermo Finnigan LCQ spectrometer. 1H NMR spectra were registered in $CDCl_3$ using spectrometers Bruker AVANCE-II-300 (300 MHz) and Bruker Avance 500 (500 MHz).

Differential Scanning Calorimetry (DSC) and Thermogravimetry Studies

Calorimetric studies were carried out with the differential heat flow calorimeter of DSC 204 F1 Phoenix "NETZSCH". All samples with mass of 10 mg were run from 263 K to 473 K in an argon atmosphere with sapphire as a standard. The heating rate of a hermetic aluminum crucible with a dried macroheterocycle was equal to 5 K/min and reproducibility of the results obtained was estimated to be within 2%. The experimental DSC signals plotted versus T are shown in Figure 1.

Thermogravimetric measurements were performed in an argon atmosphere using a Netzsch TG 209 F thermobalances. A weighted crystalline sample (3–7 mg) was placed into a platinum crucible and heated in a static atmosphere of Ar at a rate of 5 K/min in the temperature range of 298–1173 K (25–950 °C). The samples were pre-dried to constant weight in a Fisher vacuum apparatus at room temperature.

Quantum-Chemical Calculations

The geometry optimization of macrocycles **1–4** was performed using the GAUSSIAN 09 program package,^[15] the density functional method, the B3LYP hybrid functional,^[16] and the CC-pVDZ basis set.^[17] The stabilization energy of the hydrogen bonds (E_{stab} , kJ/mol) and the value of the transferred charge (q_{stab} , charge units) were calculated by means of NBO analysis.^[18,19] A full description of the calculation procedure is given in reference.^[20]

Results and Discussion

Differential Scanning Calorimetry

Figure 1 compares the DSC curves for methylpheophorbide **1** and its two glycol derivatives **2** and **3** at 263–473 K. The compound **1** is rather stiff and does not reveal any phase transitions in the temperature range studied. In contrast, two peaks have been detected for compounds **2** and **3**. It could be assumed the first one is associated with conformational changes of the glycol fragment which is absent in macrocycles **1** and **4**. The relatively low temperature of transition and its small heat effect (7 kJ/mol and lower, Figure 1) testifies that molecular packing is not too dense. The most probable reason is H-bond formation between the lone pair of oxygen atom of carbonyl group and hydrogen of glycol fragment of the neighbour molecules. These H-bonds strongly restrict the number of possible conformations of glycol fragments.

This assumption is confirmed by geometry optimization of compounds **1–4** and NBO analysis data (Table 1). Though, only glycol conjugates of methylpheophorbide **1** and chlorin **4** are able to the H-bonds $C=O \cdots H-O$ formation (Figure 2). H-bonds considered in NBO analysis are

intramolecular ones. Meantime, compounds with several H-donor and H-acceptor centers like macrocycles **2** or **3** can form some sort of intermolecular H-bonded and supramolecular structures destroyed then at about 300 K in a solid state. Namely breaking of these bonds give signals on DSC curves at low temperatures about 298 K (Figure 1). Low energy of these phase transitions is explained by weakness of H-bonds which are only about 10 kcal/mol (Table 1). Two stable conformations are found for macrocycles **2** and **3** (Table 1, Figure 2). Formation of H-bonds between oxygen atom of ester group and OH-group of glycol residue is found to be about 5 kcal/mol more favorable in the case of phorbine derivative **2** if compare to chlorin **3** (Table 1) because of better conditions for spatial preorganization of reaction centers in former macrocycle.

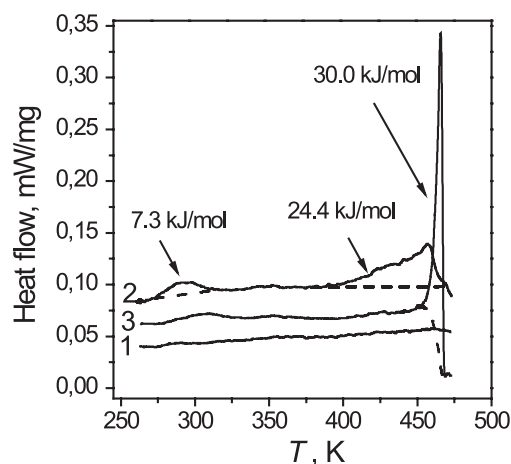


Figure 1. DSC traces for solid compounds **1–3**.

Curves are shifted from each other on 0.02 mW for better visualization. Dash lines show calculated curves for estimating the heat effects of processes occurring in a solid state. For **1** no any phase transitions have been detected; **2** reveals two peaks at $T=293$ K and $T=456$ K; **3** gives one sharp peak at $T=466$ K and smaller effect at about 303 K.

Table 1. Parameters of intramolecular H-bonds of PSs **2** and **3** calculated from NBO-analysis.

Comp.	Bond distances (Å) and angles (deg)			E_{stab} , kJ/mol (kcal/mol)	q_{stab} , e.u.
	O \cdots H	O \cdots O	O-H \cdots O		
2 (1)	1.839	2.811	172.9	63.89 (15.3)	0.028
2 (2)	1.904	2.874	172.7	49.54 (11.8)	0.024
3 (1)	1.888	2.805	155.2	39.03 (9.3)	0.016
3 (2)	1.843	2.790	161.3	62.67 (15.0)	0.028

DSC peaks for both derivatives **2** and **3** are found at higher temperatures. The compound **2** shows gradual disordering at $T>380$ K. The position of the broad peak maximum at $T=456$ K is close to the determined experimentally temperature of PS melting (445 K). Further temperature rise appears to lead to partial PS decomposition which can be seen from thermogravimetry data (Table 2)

and dealing with the loss of glycol residue and macroring destruction. The compound **3** gives a sharp peak at $T=466$ K, which is also nearly identical to experimentally determined temperature of melting (463 K). Compound **1** does not show any phase transitions at both low and high temperature of 263–473 K interval. It does not form any H-bonds and melts at 519 K only (Table 2).

Such a way, phase transitions on the DSC curves are associated with a rapid H-bonds breaking, melting and then partial decomposition of PS. It is interesting to note that the sum of two heat effects for the compounds **2** and **3** are almost equal in the temperature interval studied (Figure 1).

Thermal Stability

Investigation of thermal stability of chlorins **1-4** by thermogravimetry method (Table 2) indicates that they are quite stable in inert atmosphere. Thermograms are typical for chlorophyll derivatives and represented by two high temperature stages of aromatic chromophore destruction (Figure 2).

It should be mentioned that all compounds studied are exposed to thermodestruction after their melting points. The initial temperature of the first destruction stage is higher for pheophorbide *a* and chlorin e_6 derivatives (compounds **1** and **4**; 624 and 606 K, respectively) and decreases dramatically down to 519 K after insertion of diethylene glycol fragment into the macrocyclic molecule (Table 2).

Thus, our comparative analysis indicates that the PSs studied are stable in a wide temperature range. Introduction

of the glycol fragment into the macrocycle destabilizes PS and causes phase transitions appeared in temperature range 265–475 K. H-Bonds strongly restrict the number of possible conformations of glycol fragments for **2** and **3** and influence on solubility preventing the PS transfer from a solid state to liquid phase.

Conclusions

In this paper, we have focused our attention on the behavior of recently synthesized macrocyclic compounds which are believed to have some importance for photodynamic therapy.^[9] Our comparative analysis clearly indicates that solid potential PSs created on chlorophyll *a* platform are stable in a wide temperature range. Hydrophobic methylpheophorbide *a* **1** does not reveal any phase transitions in a solid state both at low and high temperatures. In contrast, glycol derivatives undergo phase transitions, especially, at higher temperatures which correspond to melting of the macroheterocycles. Intramolecular H-bonding is responsible for the phase transitions of glycol conjugates at lower temperatures. This conclusion is confirmed by quantum-chemical calculations.

Thermodestruction of PS in inert atmosphere takes place at temperatures higher 520 K and is represented by two step processes within 520–760 K.

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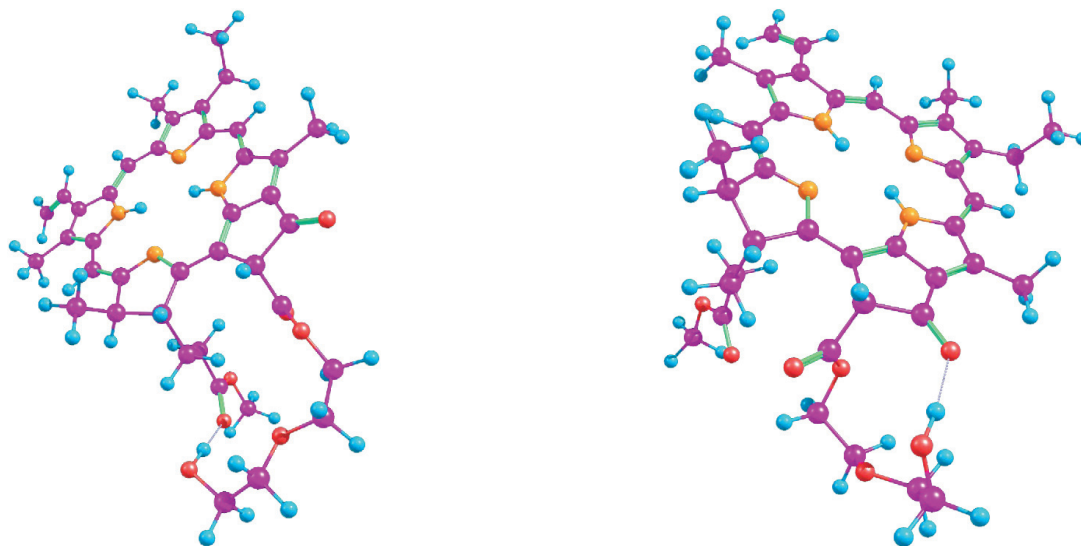


Figure 2. Geometry structure of stable conformations of compound **2**: (1) – right and (2) left, optimized by DFT method (basis CC-pVDZ).

Table 2. Thermodestruction characteristics of compounds **1**, **3** and **4** in inert atmosphere.

Comp.	Stage 1				Stage 2			
	t_{st} , K	T_{max} , K	T_{fin} , K	Δm , %	t_{st} , K	T_{max} , K	T_{fin} , K	Δm , %
1	624.17	672.02	681.91	26.6	699.40	708.15	753.60	25.3
3	519.48	573.73	595.11	20.6	645.03	693.84	755.67	39.1
4	606.44	641.33	654.53	27.2	697.77	704.05	741.58	23.2

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